

1           **Quantification of Complex Polycyclic Aromatic Hydrocarbon Mixtures in Standard**  
2                                   **Reference Materials Using GC×GC/ToF-MS**

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9           **Abstract**

10          This research is the first to quantify complex PAH mixtures in NIST SRMs using comprehensive  
11          two-dimensional gas chromatography coupled to time-of-flight mass spectrometry  
12          (GC×GC/ToF-MS), with and without extract cleanup, and reports previously unidentified PAH  
13          isomers in the NIST SRMs. We tested a novel, high orthogonality GC column combination (LC-  
14          50×NSP-35), as well as with a commonly used column combination (Rtx-5ms×Rxi-17) for the  
15          quantification of a complex mixture of 85 different PAHs, including parent (PAHs), alkyl-  
16          (MPAHs), nitro- (NPAHs), oxy- (OPAHs), thio- (SPAHS), bromo- (BrPAHs), and chloro-PAHs  
17          (CIPAHs) in extracts from two standard reference materials: NIST SRM1650b (diesel particulate  
18          matter), with cleanup and NIST SRM1975 (diesel particulate extract), with and without extract  
19          cleanup. The LC-50×NSP-35 column combination resulted in an average absolute percent  
20          difference of 33.8%, 62.2% and 30.8% compared to the NIST certified PAH concentrations for  
21          NIST SRM1650b, NIST SRM1975 with cleanup and NIST SRM1975 without cleanup, while the

22 Rtx-5ms×Rxi-17 resulted in an absolute percent difference of 38.6%, 67.2% and 79.6% for NIST  
23 SRM1650b, NIST SRM1975 with cleanup and NIST SRM1975 without cleanup, respectively.  
24 This GC×GC/ToF-MS method increases the number of PAHs detected and quantified in  
25 complex environmental extracts using a single chromatographic run. Without clean-up, 7  
26 additional compounds were detected and quantified in NIST SRM1975 using the LC-50×NSP-35  
27 column combination. These results suggest that the use of the LC-50×NSP-35 column  
28 combination in GC×GC/ToF-MS not only results in better chromatographic resolution and  
29 greater orthogonality for the separation of complex PAH mixtures, but can also be used for the  
30 accurate quantification of complex PAH mixtures in environmental extracts without cleanup.

### 31 **Keywords**

32 PAHs, Comprehensive Two-dimensional Gas Chromatography, ToF-MS, Complex  
33 Environmental Samples, Quantitation of POPs.

### 34 **1. Introduction**

35 Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants that  
36 constitute a large and diverse class of organic molecules. PAHs are of concern due to their  
37 potential persistence, bioaccumulation and toxic effects [1,2,3,4]. Some PAH derivatives are  
38 more carcinogenic and mutagenic than their parent compounds [5,6].

39 The extracts from complex environmental samples may contain a variety of PAHs with different  
40 molecular sizes and structures including: parent PAHs (PPAHs), alkylated-PAHs (MPAHs),  
41 nitro-PAHs (NPAHs), oxy-PAHs (OPAHs), thio-PAHs (SPAHS), chlorinated (CIPAHs) and  
42 brominated-PAHs (BrPAHs). The most prominent source of PPAHs and MPAHs is the

43 incomplete combustion of organic material [7,8] in either natural processes, such as forest fires,  
44 volcanic eruptions and hydrothermal processes [9,10,11,12], or anthropogenic processes, such as  
45 the combustion of fossil fuel and biomass [13,14,15]. Heterocyclic analogs of PAHs, in which  
46 one or more carbon atoms are replaced by nitrogen, sulfur, or oxygen, have also been measured  
47 as environmental contaminants. NPAHs are formed during the pyrolysis of nitrogen-containing  
48 organic materials and significant concentrations are found in industrial and urban atmospheres,  
49 tobacco smoke, engine exhaust, coal tar and coal gasification residues [8,16]. SPAHs are  
50 emitted from most of the same combustion sources as PPAHs and NPAHs [8]. Chemical  
51 oxidation and photochemical alteration represent significant sources of OPAH derivatives to the  
52 environment [17,18,19]. Waste incinerators, water chlorination facilities and automobile and  
53 diesel exhaust have been shown to form ClPAHs and BrPAHs [4,17,18,19], in addition to  
54 PPAHs, MPAHs, NPAHs, OPAHs, and SPAHs.

55 The analysis of environmental extracts containing PAHs is often complex and requires cleanup  
56 steps and multiple liquid or gas chromatographic methods. Currently, the analysis and  
57 quantification of complex PAH mixtures in environmental extracts requires three different one-  
58 dimensional GC/MS methods with a total run time of 141.6 min per sample: NPAHs, SPAHs  
59 and OPAHs method (45.7 min) [16], PPAH and MPAHs method (46 min)[16], and Cl and Br-  
60 PAH method (49.9 min) [20], in addition to the time required for sample cleanup that often  
61 includes adsorption, solid phase extraction (SPE) and gel permeation chromatography (GPC).

62 In order to reduce the analysis time of PAHs contained in a complex environmental mixture, a  
63 technique with higher chromatographic peak capacity is needed. Comprehensive two-  
64 dimensional gas chromatography (GC×GC) enhances the gas chromatographic separation of

65 complex organic mixtures [21] using two different GC columns, with different retention  
66 mechanisms, for the separation of analytes. Theoretically, the peak capacity generated by  
67 GC×GC is equal to the product of the individual peak capacities of each column used [22,23].  
68 However, in practice, the peak capacity is equal to the product of individual peak capacities,  
69 minus the cross information [24]. Therefore, a GC×GC method with high orthogonality, and low  
70 correlation of retention times between dimensions, is preferred.

71 Quantification in GC×GC/ToF-MS is a more complex process than in one-dimensional GC/MS,  
72 where in the latter case a single retention time and peak response are associated with each  
73 analyte in the extract. In GC×GC/ToF-MS, a series of modulated peaks (sub-peaks) are  
74 generated and detected, and the retention time and response are represented by a distribution of  
75 values generated by this process [25,26]. Quantification in GC×GC/ToF-MS is an extension of  
76 one-dimensional GC/MS in that these individual sub-peak areas are added together [27]. With  
77 both GC/MS and GC×GC/ToF-MS, an increase in quantification error occurs because of  
78 inaccurate determination of the peak baseline and incorrect identification of peak start and end  
79 times, as well as tailing, fronting and overloading of each modulated peak [28]. However, these  
80 errors are compounded in GC/MS because of its decreased chromatographic resolution compared  
81 to GC×GC/ToF-MS. Peak tailing, fronting and overloading are especially important with  
82 GC×GC/ToF-MS because of the shorter and narrower second dimension column. In addition,  
83 small variations in integration parameters for the modulated peaks produce variable  
84 quantification results with a GC×GC system [29].

85 Previously, we reported greater separation of complex PAH mixtures in GC×GC/ToF-MS using  
86 a liquid crystal column (LC-50) in the first dimension and a nano-stationary phase column (NSP-

87 35) in the second dimension due to its higher orthogonality than the commonly used combination  
88 (Rtx-5ms×Rxi-17) [30]. The objective of this research was to determine if this novel, high  
89 orthogonality column combination (LC-50×NSP-35), as well as the traditional column  
90 combination (Rtx-5ms×Rxi-17), resulted in reliable and reproducible quantification of a complex  
91 mixture of 85 different PAHs, including PPAHs, MPAHs, NPAHs, SPAHs, OPAHs, BrPAHs  
92 and ClPAHs, in two National Institute of Standards and Technology (NIST) standard reference  
93 materials (SRM), with and without cleanup. PAHs were quantified in NIST SRM1650b (diesel  
94 particulate matter) with silica gel solid phase extraction (SPE) cleanup and in NIST SRM1975  
95 (diesel particulate extract) with and without silica gel SPE cleanup, using both column  
96 combinations. The ratio of the summation of the three most intense modulated peaks for each  
97 target PAH to the three most intense modulated peak of its corresponding surrogate  
98 perdeuterated PAH was used to overcome the quantification problems in atmospheric extracts  
99 (PM<sub>2.5</sub>) described above [25,26]. This research is the first to quantify complex PAH mixtures in  
100 NIST SRMs using GC×GC/ToF-MS, with and without extract cleanup, and reports previously  
101 unidentified PAH congeners in the NIST SRMs.

## 102 **2. Materials and Methods**

### 103 **2.1. Chemicals and Reference Materials**

104 The standard reference materials, SRM1975 and SRM1650b, were purchased from NIST (NIST,  
105 Gaithersburg, MD) [31]. Standard solutions of 18 PPAHs were purchased from ChemService  
106 (West Chester, PA, USA), standard solutions of 9 MPAHs, 18 NPAHs and 2 SPAHs were  
107 purchased from AccuStandard (New Haven, CT, USA), and neat standards of 17 OPAHs were  
108 purchased from Sigma-Aldrich (St. Louis, MO, USA). Standard solutions of 15 ClPAHs and 6

109 BrPAHs were synthesized by Dr. Takeshi Ohura from the University of Shizuoka in Shizuoka,  
110 Japan, using published procedures [20,32,33]. The entire list of PAH analytes can be found in  
111 Table S-1. Isotopically labeled PAHs, OPAHs, and NPAHs were purchased from CDN Isotopes  
112 (Point-Clare, Quebec, Canada) and Cambridge Isotopes Laboratories (Andover, MA) and  
113 included d<sub>6</sub>-1,4-naphthaquinone, d<sub>4</sub>-1,4-benzoquinone, d<sub>10</sub>-fluorene, d<sub>7</sub>-1-nitronaphthalene, d<sub>10</sub>-  
114 phenanthrene, d<sub>8</sub>-anthraquinone, d<sub>9</sub>-5-nitroacenaphthene, d<sub>10</sub>-pyrene, d<sub>9</sub>-9-nitroanthracene, d<sub>12</sub>-  
115 triphenylene, d<sub>9</sub>-3-nitrofluoranthene, d<sub>9</sub>-1-nitropyrene, d<sub>12</sub>-benzo[a]pyrene, d<sub>11</sub>-6-nitrochrysene,  
116 d<sub>12</sub>-benzo[ghi]perylene as surrogates and d<sub>10</sub>-acenaphthene, d<sub>8</sub>-9-fluorenone, d<sub>10</sub>-fluoranthene,  
117 d<sub>12</sub>-benzo[k]fluoranthene, d<sub>9</sub>-2-nitrobiphenyl, d<sub>9</sub>-2-nitrofluorene as internal standards.

## 118 **2.2. Sample Preparation**

119 Three aliquots of NIST SRM1650b and NIST SRM1975 were spiked with known amounts of  
120 labeled PAH, OPAH and NPAH surrogates prior to sample preparation. NIST SRM1650b was  
121 extracted using a method based on pressurized liquid extraction (PLE) with dichloromethane  
122 (DCM) that has been previously described [34,35,36]. The resulting NIST SRM1650b extracts  
123 and the NIST SRM1975 aliquots were cleaned up using 20 g silica gel columns (Mega BE-SI,  
124 Agilent Technologies, New Castle, DE) and eluted in three fractions, with 100 % hexane (non-  
125 polar fraction) , 100 % DCM (fraction containing PAHs) and 100 % ethyl acetate (polar  
126 fraction). The DCM fraction was then concentrated to 300 µL under a gentle stream of N<sub>2</sub> using  
127 a Turbovap II (Caliper Life Sciences, MA), solvent exchanged to ethyl acetate and spiked with  
128 known amounts of internal standards prior to analysis. An aliquot of NIST SRM1975, without  
129 cleanup, was also spiked with surrogates and internal standards prior to analysis.

## 130 **2.3. GC×GC/ToF-MS Quantification**

131 A GC×GC/ToF-MS Pegasus 4D (Leco, St. Joseph, MI, USA) was used for this study. The  
132 instrument consisted of an Agilent 6890 gas chromatograph (Palo Alto, CA, USA) with a  
133 secondary oven, a split/splitless injector, and a non-moving quad-jet dual stage modulator. The  
134 two GC columns in the system were connected using an Agilent CPM union (part no. 188-5361)  
135 for 0.1-0.25 mm I.D. columns. Two GC column combinations were used. Column combination  
136 “A” was a low-polarity Rtx-5ms column (35 m × 0.25 mm × 0.25 μm) (Restek, Bellefonte, PA,  
137 USA) with a 5 m guard column, followed by a mid-polarity Rxi-17 column (1.2 m × 0.10 mm ×  
138 0.10 μm) (Restek, Bellefonte, PA, USA). Column combination “B” was a liquid crystal LC-50  
139 column (10 m × 0.15 mm × 0.10 μm) (J&K Scientific, Edwardsville, Nova Scotia, Canada),  
140 followed by a nano-stationary phase NSP-35 column (1.2 m × 0.10 mm × 0.10 μm) (J&K  
141 Scientific, Edwardsville, Nova Scotia, Canada). The data processing was performed using  
142 ChromaTOF version 4.33. The optimization of both column combinations has been previously  
143 described [30]. However, the modulation time was changed from 5 to 7 s in order to increase  
144 peak height and instrument sensitivity for some of the PAHs measured.

145 Five-point calibration curves, ranging from 5-1000 pg/μL, were used for quantification, with  
146 concentration ranges varying slightly among the different PAHs. A complete description of the  
147 concentrations in the calibration curves can be found in Table S-2. An internal standard  
148 concentration of 250 pg/μL was used for all calibration standard solutions.

149 Selected modulated peaks were used for PAH quantification rather than full sub-peak  
150 summation, to reduce quantitation time [26,28]. We calculated the ratio of the peak area for the  
151 three most intense modulated sub-peaks of each PAH to the peak area for the three most intense  
152 modulated sub-peaks of the respective deuterated PAH surrogate to reduce errors associated with

153 the partial peak area [25]. Three modulated sub-peaks were used for both in-phase and out-of-  
154 phase peaks [28]. The GC×GC/ToF-MS operating conditions can be found in Table S-3.

155 Each SRM was analyzed and quantified in triplicate ( $n = 3$ ) and the 95% confidence interval of  
156 the concentration calculated. All PAH concentrations reported correspond to S/N ratio greater  
157 than 10 times the standard deviation of the detected noise and the limits of quantitation ranged  
158 from 3 pg/μL for PPAH to 18 pg/μL for NPAH.

159

### 160 **3. Results and Discussion**

#### 161 **3.1. Quantification of NIST SRM1650b (Diesel Particulate Matter) with Cleanup**

162 Figure S-1 shows the total ion chromatogram (TIC) for the analysis of the NIST SRM1650b  
163 extract, after silica gel SPE cleanup, using column combinations “A” (Rtx-5ms×Rxi-17) (Figure  
164 S-1A) and “B” (LC-50×NSP-35) (Figure S-1F). The separation patterns observed for both  
165 column combinations were consistent to what was reported previously, showing a predictable  
166 elution pattern due to the strong correlation between the separation mechanisms in combination  
167 “A”, and a more random elution pattern in combination “B” due to its higher orthogonality [30].

168 Co-elutions among PAHs, and with the unresolved complex mixture (UCM), were evident when  
169 column combination “A” was used (Figures S-1C, S-1D and S-1E). With column combination  
170 “B”, the UCM eluted at earlier retention times in the first dimension and was distributed  
171 throughout the second dimension retention times compared to column combination “A”. This  
172 resulted in better separation of the PAHs from the UCM, especially for the later eluting PAHs  
173 (Figures S-1H, S-1I and S-1J) [30].



174 Figure 1 shows the scatter plots of the NIST certified and reference PAH concentrations for  
175 NIST SRM1650b [31] versus the PAH concentrations determined by GC×GC/ToF-MS using  
176 column combinations “A” (Figure 1A) and “B” (Figure 1B). The 1:1 line is shown in each plot,  
177 along with the 95% confidence interval (CI) (shown with error bars), for triplicate measurements  
178 ( $n = 3$ ).

179 Table S-4 shows the average measured PAH concentrations for NIST SRM1650b using column  
180 combinations “A” and “B” with GC×GC/ToF-MS, the relative standard deviation (RSD), and the  
181 95% confidence interval (CI) for the triplicate measurements ( $n = 3$ ), as well as the NIST  
182 certified concentrations. The RSDs for the measured PAH concentrations for NIST SRM1650b  
183 were, on average, 11.2% for column combination “A” and 11.9% for column combination “B”.

184 The percent difference between PAH concentrations determined by GC×GC/ToF-MS and the  
185 NIST certified PAH concentrations (shown in Table S-4 and Table S-5) was calculated using  
186 equation (1):

$$187 \quad \Delta[PAHs] = \frac{[PAHs]_{exp} - [PAHs]_{NIST}}{[PAHs]_{NIST}} \times 100 \quad (1)$$

188 Where  $\Delta[PAHs]$  is the percent change of PAHs relative to the NIST certified concentrations,  
189  $[PAHs]_{exp}$  is the PAH concentrations determined by GC×GC/ToF-MS and  $[PAHs]_{NIST}$  is the  
190 NIST certified PAH concentrations. For NIST SRM1650b,  $\Delta[PAHs]$  ranged from -65.1% for  
191 naphthalene to 340.5% for dibenzo[a,h]anthracene, with an average  $|\Delta[PAHs]|$  of 38.6%, when  
192 column combination “A” was used (Table S-4).  $\Delta[PAHs]$  ranged from -50.8% for anthracene to  
193 259.5% for dibenzo[a,h]anthracene, with an average  $|\Delta[PAHs]|$  of 33.8%, when column

194 combination “B” was used (Table S-4, NIST SRM1650b). Combination “B” resulted in PAH  
195 concentrations that were slightly closer to the NIST certified PAH concentrations for NIST  
196 SRM1650b (33.8% average absolute percent difference) compared to combination “A” (38.6%  
197 average absolute percent difference). Dibenz[a,h]anthracene (DahA) had the highest  $|\Delta[PAHs]|$   
198 in both column combinations due to its partial co-elution with indeno[1,2,3-c,d]pyrene in column  
199 combination “A”, peak broadening in column combination “B”, and its relatively low  
200 concentration in NIST SRM1650b.

201 A two-sample student t-test was used to determine if the average measured PAH concentrations  
202 for NIST SRM1650b were statistically different from the NIST certified PAH concentrations.  
203 Out of the 20 PAHs that were measured in NIST SRM1650b and had certified values, 11 of the  
204 20 PAHs had measured concentrations that were statistically different ( $p < 0.05$ ) from the NIST  
205 certified concentration when column combination “A” was used and 6 of the 20 PAHs had  
206 measured concentrations that were statistically different ( $p < 0.05$ ) when column combination  
207 “B” was used (Table S-4). This suggests that column combination “B” can be used for the  
208 quantitation of complex PAH mixtures with greater accuracy than combination “A”.

209 Naphthalene (NAP), 1-methylnaphthalene (1 met NAP) and 2-methylnaphthalene (2 met NAP)  
210 showed weak interaction with the liquid crystal column used in column combination “B”, eluting  
211 with the solvent peak in less than 5 minutes, and are reported as not detected (n.d.) in Table S-4  
212 and Table S-1. In addition, peak tailing was observed in sub-peaks of polar PAHs, including  
213 some OPAHs and SPAHs with both column combinations. A similar behavior has been  
214 previously reported in the literature and was described as an effect coming from excessive cold  
215 jet flow modulation; where the hot jet was not able to efficiently launch all of the analyte mass

216 that was trapped in the modulator into the second dimension column, increasing the number of  
217 sub-peaks generated for each compound and decreasing the S/N ratio [37]. In our experiment,  
218 this behavior represented a source of error in both column combinations for the quantification of  
219 PAHs such as dibenzothiophene (Dibenzth), 9,10-anthraquinone (9,10 ANTq), 9-fluorenone (9  
220 Fluo), 9-chlorophenanthrene (9 Cl PHE) and phenanthrene-1,4-dione (1,4 PHEq).

221 Eighty-six PAHs (32 PPAHs, 31 MPAHs, 22 NPAHs and 1 SPAHs) had NIST certified or  
222 reference concentrations for NIST SRM1650b based on their measurement using one-  
223 dimensional GC/MS with three different stationary phases [31]. Of the 85 different PAHs in our  
224 compound list (Table S-1), 47 PAHs (17 PPAHs, 8 MPAHs, 8 NPAHs, 1 SPAH, 10 OPAHs and  
225 3 CIPAHs) were measured by GC×GC/ToF-MS using column combination “A” and 49 PAHs  
226 (17 PPAHs, 7 MPAHs, 10 NPAHs, 2 SPAHs, 10 OPAHs, 1 BrPAH, 2 CIPAHs) using column  
227 combination “B”. The PAH concentration profiles measured in NIST SRM1650b are shown in  
228 Figures 2A and 2B for column combinations “A” and “B”, respectively. These profiles include  
229 PAHs that were not previously reported by NIST, including 1,3-dimethylnaphthalene, 2,6-  
230 dimethylnaphthalene, retene, 2-nitroanthracene, 2-nitropyrene, 3-nitrodibenzofuran, 4-  
231 nitrobiphenyl, 2-nitrodibenzothiophene, 1,4-anthraquinone, 1,4-naphthaquinone, 2-methyl-9,10-  
232 anthraquinone, 5,12-naphthacenequinone, 9,10-anthraquinone, 9,10-phenanthrenequinone, 9-  
233 fluorenone, benz[a]anthracene-7-12-dione, benzanthrone, benzo[a]fluorenone,  
234 benzo[c]phenanthrene-[1,4]-quinone, phenanthrene-1,4-dione, 2-bromofluorene, 1-chloropyrene,  
235 2-chloroanthracene, 9-chloroanthracene and 9-chlorophenanthrene (Table S-1). The  
236 measurement of this NIST SRM by GC×GC/ToF-MS results in a more comprehensive  
237 determination of its complex PAH mixture, without significant increase in analysis time,  
238 compared to one-dimensional GC/MS.

### 239 3.2. Quantification of PAHs in NIST SRM1975 (Diesel Particulate Extract) with Cleanup

240 Figure S-2 shows the TIC for the analysis of the NIST SRM1975 extract, after silica gel SPE  
241 cleanup, using column combinations “A” (Figure S-2A) and “B” (Figure S-2F). The elution  
242 pattern observed was also consistent with what was previously reported [30]. Co-elutions among  
243 the PAHs and the UCM were evident with column combination “A” in Figures S-2C, S-2D and  
244 S-2E. When column combination “B” was used (Figure S-2F), the PAHs eluted in a more  
245 random pattern compared to column combination “A”, with better resolution and separation from  
246 the UCM, especially for the late eluting PAHs (Figures S-2H, S-2I and S-2J).

247 Figure 1 shows the scatter plots for the NIST certified and reference PAH concentrations for  
248 NIST SRM1975 versus the concentrations determined by GC×GC/ToF-MS using column  
249 combinations “A” (Figure 1C) and “B” (Figure 1D). For the NIST SRM1975 extract after  
250 cleanup, column combination “B” resulted in PAH concentrations that were slightly closer to the  
251 NIST certified PAH concentrations (62.2% absolute percent difference) compared to column  
252 combination “A” (67.2% absolute percent difference).

253 Table S-5 shows the average measured PAH concentrations for NIST SRM1975 using column  
254 combinations “A” and “B” with GC×GC/ToF-MS, as well as the NIST certified PAH  
255 concentrations. The RSDs for the measured PAH concentrations for NIST SRM1975 after  
256 cleanup were, on average, 9.28% for column combination “A” and 16.6% for column  
257 combination “B” ( $n = 3$ ).

258 Equation 1 was used to evaluate the percent difference between PAH concentrations determined  
259 by GC×GC/ToF-MS and the NIST certified PAH concentrations. For NIST SRM1975 after

260 cleanup,  $\Delta[PAHs]$  ranged from -14.1% for fluoranthene to 494.1% for benzo[k]fluoranthene,  
261 with an average  $|\Delta[PAHs]|$  of 67.2% when column combination “A” was used (Table S-5).  
262  $\Delta[PAHs]$  ranged from -16.3% for fluoranthene to 196.2% for benzo[b]fluoranthene, with an  
263 average  $|\Delta[PAHs]|$  of 62.2%, when column combination “B” was used (Table S-5). Out of the  
264 11 PAHs that were measured in NIST SRM1975 and had certified values, 5 of the PAHs had  
265 measured concentrations that were statistically different ( $p < 0.05$ ) from the NIST certified  
266 concentrations when column combination “A” was used and 6 of the PAHs had measured  
267 concentrations that were statistically different ( $p < 0.05$ ) when column combination “B” was  
268 used (Table S-5).

269 Fifty-seven PAHs (18 PPAHs, 20 MPAHs and 19 NPAHs) had NIST certified or reference  
270 concentrations for NIST SRM1975 based on their measurement using one-dimensional GC/MS  
271 with two different stationary phases [31]. Of the 85 different PAHs in our compound list (Table  
272 S-1), 55 PAHs (17 PPAHs, 7 MPAHs, 13 NPAHs, 1 SPAH, 11 OPAHs and 6 CIPAHs) were  
273 measured by GC×GC/ToF-MS using column combination “A” and 46 PAHs (16 PPAHs, 5  
274 MPAHs, 11 NPAHs, 2 SPAHs, 9 OPAHs and 3 CIPAHs) using column combination “B”. The  
275 PAH concentration profiles measured in NIST SRM1975, including those not reported by NIST  
276 (acenaphthene, acenaphthylene, anthracene, benzo[a]pyrene, 1,3-dimethylnaphthalene, 1-  
277 methylpyrene, 2,6-dimethylnaphthalene, retene, 1-nitronaphthalene, 2-nitrofluoranthene, 2-  
278 nitrofluorene, 2-nitronaphthalene, 2-nitropyrene, 3-nitrobiphenyl, 3-nitrofluoranthene, 3-  
279 nitrophenanthrene, 7-nitrobenz[a]anthracene, 9-nitrophenanthrene, 2-nitrodibenzothiophene,  
280 dibenzothiophene, 2-methyl-9,10-anthraquinone, 4H-cyclopenta[def]phenanthrene-4-one, 5,12-  
281 naphthacenequinone, 9,10-phenanthrenequinone, 9-fluorenone, acenaphthenequinone,  
282 benz[a]anthracene-7,12-dione, benzanthrone, benzo[a]fluorenone, benzo[c]phenanthrene-1,4-

283 quinone, phenanthrene-1,4-dione, 1-chloropyrene, 2-chloroanthracene, 3-chlorofluoranthene, 8-  
284 chlorofluoranthene, 9-chloroanthracene, 9-chlorophenanthrene) are shown in Figures 2C and 2D  
285 for column combinations “A” and “B”, respectively.

### 286 **3.3. Quantification of PAHs in NIST SRM1975 (Diesel Particulate Extract) without** 287 **Cleanup**

288 To further test the use of GC×GC/ToF-MS to measure complex mixtures of PAHs in complex  
289 environmental matrices, a 225 μL aliquot of the NIST SRM1975 extract, without cleanup, was  
290 spiked with 75 μL of both internal standard and surrogate solutions. This extract was analyzed in  
291 triplicate using GC×GC/ToF-MS with column combinations “A” and “B”.

292 Figures 3A and 3F show the TICs for the analysis of the NIST SRM1975 extract without cleanup  
293 using column combinations “A” and “B”, respectively. The PAHs eluted in a similar pattern to  
294 the cleaned extract (Figures S-2A and S-2F). However, Figures 3C, 3D and 3E show that the  
295 UCM co-eluted and interfered with some of the PAHs when column combination “A” was used,  
296 while Figure 3F shows that the majority of the UCM eluted in the first 15 minutes and did not  
297 co-elute with the PAHs when column combination “B” was used (Figures 3I and 3J).

298 Figure 1 shows the scatter plots of the NIST certified and reference PAH concentrations in NIST  
299 SRM1975 versus the concentrations determined by GC×GC/ToF-MS in NIST SRM1975 without  
300 cleanup using column combinations “A” (Figure 1E) and “B” (Figure 1F). For the NIST  
301 SRM1975 extract without cleanup, column combination “B” resulted in PAH concentrations that  
302 were closer to the NIST certified PAH concentrations for NIST SRM1975 (30.8% absolute  
303 percent difference) compared to combination “A” (79.6% absolute percent difference).

304 Table S-5 shows the average measured PAH concentrations for NIST SRM1975 (without  
305 cleanup) using column combinations “A” and “B” with GC×GC/ToF-MS, as well as the NIST  
306 certified PAH concentrations. The RSDs for the measured PAH concentrations for NIST  
307 SRM1975 without cleanup were, on average, 27.1% for column combination “A” (higher  
308 compared to the NIST SRM1975 extract with cleanup using column combination “A”) and  
309 16.6% for column combination “B” (comparable to the NIST SRM1975 extract with cleanup  
310 using column combination “B”) ( $n = 3$ ).

311 For NIST SRM1975 without cleanup,  $\Delta[PAHs]$  ranged from -43.3% for fluoranthene to 335.3%  
312 for benzo[k]fluoranthene, with an average  $|\Delta[PAHs]|$  of 79.6%, when column combination “A”  
313 was used (Table S-5).  $\Delta[PAHs]$  ranged from -40.0% for 6-nitrochrysene to 70.42% for  
314 benzo[e]pyrene, with an average  $|\Delta[PAHs]|$  of 30.8%, when column combination “B” was used  
315 (Table S-5). Out of the 11 PAHs that were measured in the NIST SRM1975 extract without  
316 cleanup and had NIST certified values, 6 of the PAHs had measured concentrations that were  
317 statistically different ( $p < 0.05$ ) from the NIST certified values when column combination “A”  
318 was used and 4 of the PAHs had measured concentrations that were statistically different ( $p <$   
319  $0.05$ ) when column combination “B” was used (Table S-5). This suggests that column  
320 combination “B” can be used for an accurate quantitation of complex PAH mixtures in samples  
321 with reduced or no cleanup.

322 Fifty-seven PAHs (18 PPAHs, 20 MPAHs and 19 NPAHs) had NIST certified or reference  
323 concentrations for NIST SRM1975 based on their measurement using one-dimensional GC/MS  
324 with two different stationary phases [31]. Of the 85 different PAHs in our compound list (Table  
325 S-1), 50 PAHs (17 PPAHs, 5 MPAHs, 11 NPAHs, 1 SPAH, 14 OPAHs and 2 ClPAHs) were

326 measured by GC×GC/ToF-MS using column combination “A” (less than the 55 PAHs identified  
327 in the NIST SRM1975 cleaned extract) and 53 PAHs (17 PPAHs, 6 MPAHs, 13 NPAHs, 2  
328 SPAHs, 12 OPAHs and 3 CIPAHs) were measured using column combination “B” (more than  
329 the 46 PAHs identified in the NIST SRM1975 cleaned extract). Some of the compounds detected  
330 in extracts without cleanup, that were not detected in the cleaned extracts, included relatively  
331 polar compounds compared to the other PAHs detected (3-dibenzofuran, 4-nitrbiphenyl, 1,4-  
332 anthraquinone, 1,4-naphthaquinone, acenaphthenequinone, aceanthrenequinone and  
333 benzo[cd]pyrenone). This suggests that these more polar PAHs may be lost during the sample  
334 cleanup process.

#### 335 **4. Conclusions**

336 The analyses of NIST SRMs by GC×GC/ToF-MS resulted in a more comprehensive  
337 determination of its complex PAH composition, without a significant increase in analysis time  
338 compared to the multiple one-dimensional GC/MS methods that would be needed to target a  
339 similar number of PAHs. The quantitation results suggest that the use of column combination  
340 “B” (LC-50×NSP-35) not only resulted in better resolution and greater orthogonality for the  
341 separation of complex PAH mixtures compared to column combination “A” (Rtx-5ms×Rxi-17)  
342 [30], but also resulted in the accurate quantification of complex PAH mixtures in environmental  
343 samples containing 50 or more PPAHs, MPAHs, OPAHs, SPAHs, NPAHs, CIPAHs and  
344 BrPAHs in a single chromatographic run, and including extracts with minimal or no extract  
345 cleanup. This research is the first to quantify complex PAH mixtures in NIST SRMs using  
346 GC×GC/ToF-MS, with and without extract cleanup, using a high orthogonality column  
347 combination (LC-50×NSP-35), and reports previously unidentified PAH congeners in both NIST



348 SRMs, including OPAHs, ClPAHs, NPAHs and MPAHs using only one chromatographic run,  
349 with a significant reduction in analysis time compared to one dimensional methods.

## 350 **5. Associated Content**

351 Supporting Information: This material is available free of charge via the Internet at  
352 <http://pubs.acs.org>.

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## Figure Captions

**Figure 1:** NIST certified and reference PAH concentrations compared to concentrations determined by GC×GC/ToF-MS in: (A) NIST SRM1650b with column combination “A”, (B) NIST SRM1650b with column combination “B”, (C) cleaned NIST SRM1975 with column combination “A”, (D) cleaned NIST SRM1975 with column combination “B”, (E) NIST SRM1975 without cleanup with column combination “A”, (F) NIST SRM1975 without cleanup with column combination “B”. The lines in the plots represent a slope=1 and y-intercept=0. The inner boxes show the low concentration PAH regions (see axes).

**Figure 2:** Average concentrations of PAHs determined by GC×GC/ToF-MS in: (A) NIST SRM1650b (diesel particulate matter) with column combination “A”, (B) NIST SRM1650b (diesel particulate matter) with column combination “B”, (C) NIST SRM1975 (diesel particulate extract) with column combination “A”, (D) NIST SRM1975 (diesel particulate extract) with column combination “B”. The error bars represent the 95% CI for each triplicated measurement (n=3)

**Figure 3:** GC×GC/ToF-MS TICs for NIST SRM1975 (diesel particulate extract) without cleanup (A) using column combination “A”, (B) black and white version of A, (C), (D), (E) portions of chromatogram shown in A, (F) using column combination “B”, (G) black and white version of F, and (H), (I), (J) portions of chromatogram shown in F.